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QUERY CONTROL FORM			RTIS USE ONLY
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a. Serial No.	f. Foreign Priority	k. Print Claim(s)	p. PTO-1449
b. Applicant(s)	g. Disclaimer	l. Print Fig.	q. PTOL-85b
c. Continuing Data	h. Microfiche Appendix	m. Searched Column	r. Abstract
d. PCT	i. Title	n. PTO-270/328	s. Sheets/Figs
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SPECIFICATION

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after the freeze/thaw step, one set of duplicate samples was spun at 14,000 rpm in a microfuge to pellet the resin. The supernatant was decanted and used in the RT-PCR reaction. The other set of reactions were not microfuged (the resin was carried over into the RT-PCR reaction). Experimental conditions are shown in Table 6. Each reaction condition was performed at each cell concentration given in Table 5.

Each sample (the entire 10 µl) was then added to RT-PCR reactions using the Access RT-PCR kit and a bcr/abl primer pair (forward: 5' GGAGCTGCAGATGCTGACCAAC 3'; SEQ ID NO:3 and reverse: 5' TCAGACCCTGAGGCTCAAAGTC 3'; SEQ ID NO:4). The final concentration of reaction components was: 1X RT-PCR buffer, 200 µM each dNTP, 2 mM $MgSO_4$, 50 pmoles forward and reverse bcr/abl primers, 5 units AMV RT, and 5 U *Tfl* DNA polymerase. The reactions were cycled using the following parameters: 45 min at 48°C; 2 min at 95°C; 40 cycles of 94°C for 30 sec, 60°C for one min, and 72°C for 1 min; 1 cycle of 7 min at 72°C, and then stored at 4°C. Aliquots of each reaction (6 µl) were then analyzed on a 1.8% agarose/1X TAE gel and visualized with ethidium bromide staining.

Results are shown in Table 6. The addition of RNASIN ribonuclease inhibitor freeze medium to the cells during lysis allowed for the sensitive detection of the bcr/abl signal, down to as low as 1 cell, with increasing signal intensity with increasing cell number. The addition of 1 µl, but not 3 µl of the poly-G resin allowed detection of the bcr/abl signal down to approximately 1 to10 cells (with or without spin). The signal in the presence of polyG was weaker than with RNASIN. Addition of either 1 µl or 3 µl of PolyI allowed detection to a level comparable to RNASIN, and provided an increased level of sensitivity when not removed prior to PCR reaction. The polyI and polyG combination of resin did not result in a substantial increase in sensitivity. The results indicate that RNA polymers can replace RNASIN in single-tube, whole cell RT-PCR.

B. Two Step RT-PCR

Two-step RT-PCR reactions were also performed on the previously described K562 cell samples. The cell sample dilutions were added to 6.75 µl nuclease-free water, 1 µl oligo-dT (0.5µg), 5 µl 5X MMLV RT buffer, 1.25 µl 10mM dNTP mix, and 1 µl (50 U) MMLV H- RT point mutant in a final reaction volume of 25 µl. The reactions were incubated at